

Amendments to the Specification

Please insert the attached Abstract as the last page of the specification.

Please amend the following paragraph at page 17, lines 3-13:

Another example of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and the BLAST 2.0 algorithm, which are described in Altschul et al., *J. Mol. Biol.* 215:403-410, 1990 and Altschul et al., *Nucleic Acids Res.* 25:3389-3402, 1977. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>). The BLASTN program (for nucleotide sequences) uses as defaults a word length (W) of 11, alignments (B) of 50, expectation (E) of 10, M=5, N=-4, and a comparison of both strands. The BLASTP program (for amino acid sequences) uses as defaults a word length (W) of 3, and expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915, 1989).

Please amend the following paragraph at page 44, line 23 through page 45, line 2:

PCR fragments were digested with Nde I and Hind III restriction enzyme and cloned into the Nde I-Hind III site in the expression vector (~~Brinkman~~Brinkmann et al., *Proc. Natl. Acad. Sci. USA* 88: 8616-8620, 1991). Concerning the making of dsFv, the positions of disulfides for the stabilization of B3(Fv) were—~~??ORIGINALLY~~ identified using computer-modeled structure of B3(Fv), generated by mutating and energy minimizing the amino acid sequence and structure of McPC603, as described previously (~~Brinkman~~Brinkmann et al., *Proc. Natl. Acad. Sci. USA* 90:7538-7542, 1993). The amino acid sequences of 8H9(Fv) was simply aligned with that of B3(Fv) to determine the positions to insert cysteine residues. For the construction of 8H9(dsFv) fragments, cysteine residues were introduced in the V_H and V_L using PCR as previously described (Reiter et al., *Biochemistry* 33: 5451-5459, 1994). The following primers were used for making the dsFv;

Please amend the following paragraph at page 45, line 29 through page 46, line 9:

Cytotoxicity Assay: The specific cytotoxicity of each IT was assessed by inhibition of protein synthesis by cells exposed to various concentrations of IT. Protein synthesis was measured as cellular incorporation of ^3H -leucine (~~Brinkmann~~ Brinkmann et al., *Proc. Natl. Acad. Sci. USA* 88: 8616-8620, 1991; Onda et al., *J. Immunol.* 163:6072-6077, 1999). Cells, at a concentration of (1.6×10^4) cells/well, were plated in 96-well plates and incubated overnight. IT was diluted in PBS/0.2% BSA to desired concentrations and was added to the target cells in triplicate. The cells were incubated for 20 hours at 37°C , before the addition of $2 \mu\text{Ci } ^3\text{H}$ -leucine per well and further incubation for 2 hours at 37°C . Cells were frozen, thawed and harvested onto glass fiber filter mats using automated harvester. The radioactivity associated with the cells was counted in an automated scintillation counter. For competition experiments, excess 8H9 MAb or T6 MAb was added 15 minutes before the addition of the IT (15.5 ng/ml).

Please amend the following paragraph at page 48, lines 1-18:

This procedure not only increases stability but often has the further advantage of increasing recombinant protein yield (Reiter et al., *Nat. Biotechnol.* 14:1239-12454, 1996). Both types of immunotoxins were produced in *E. coli* and purified by ion exchange and size exclusion chromatography after renaturation from inclusion bodies as previously described (Onda et al., *J. Immunol.* 163: 6072-6077, 1999). Each RIT eluted as a monomer upon TSK gel filtration chromatography and each migrated as a single band of about 62 kDa in SDS/PAGE (Fig. 1). Immunotoxin 8H9(scFv)-PE38 was prepared from a 1 liter culture of *E. coli*. After extensive washing 100 mg of inclusion body protein was recovered that was used to make immunotoxin. The final yield was 1.7 mg or 1.7 %. In contrast 8H9(dsFv)-PE38 is prepared by combining inclusion body protein from cells grown separately that express the V_L protein or the V_H -PE38 protein. When 33 mg of V_L protein was combined with 67 mg of V_H -PE38 protein, 16 mg of purified immunotoxin was recovered, or a 16% yield (Table 1) (Buchner et al., *Anal. Biochem.* 205: 263-270, 1992). Because of this high yield, the dsFv molecule was selected for further pre-clinical development. DNA encoding V_L protein and the V_H -PE38 protein were

deposited with the American Type Culture Collection (ATCC) as Accession Nos. PTA-5661 and PTA-5660, respectively, in accordance with the Budapest treaty on November 24, 2003.